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GENETICS AND OSTEOPOROSIS

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Synopsis

Osteoporosis is a common disease characterised by reduced bone mass and increased risk of fracture. Genetic factors play an important role in regulating bone mass, bone turnover and bone geometry and also contribute to the pathogenesis of fracture by mechanisms independent of effects on bone density. In this article, we review the techniques that have been used to identify genes that regulate susceptibility to osteoporosis and discuss the major candidate genes which have been implicated in the regulation of bone mass and susceptibility to osteoporotic fracture.

Introduction

Genetic factors play an important role in the pathogenesis of osteoporosis. The importance of genetic factors in regulating susceptibility to osteoporosis is highlighted by the fact that a positive family history of hip fracture is a strong risk factor for low bone mineral density (BMD) and osteoporotic fracture (1;2). Although fracture is the most important clinical complication of osteoporosis, most studies of genetics have focussed on BMD since this is a highly heritable trait (3-6) and an important clinical predictor of osteoporotic fracture risk (7). Nonetheless, evidence of significant genetic effects on other key determinants of osteoporotic fracture risk such as quantitative ultrasound properties of bone (8), femoral neck geometry (8), muscle strength (9), bone turnover markers (10) and body mass index (11) have also been reported. There is much less information on the heritability of fracture which is the most important clinical consequence of osteoporosis. Some investigators found little evidence to suggest that fractures are heritable in the elderly (12), whereas other researchers reported that wrist fracture had a significant genetic component with heritability estimates in the range 25%-35% (13;14). Interestingly these studies showed that susceptibility to wrist fracture seemed to be largely independent of BMD. Other work has shown that the heritability of fracture is quite high in younger patients but falls off quite rapidly with age to almost zero in the elderly (15) which would explain the discrepancies between the studies cited above. Bone mineral density and the other traits mentioned above are regulated by multiple genes and their interaction with environmental factors.

Genetic approaches to identification of osteoporosis susceptibility genes

Three main approaches have been implemented in the identification of genes that predispose to osteoporosis: linkage analysis in pedigrees, experimental crosses in model animals and association studies in human populations. The basic principle of these methods and how they were utilised in the identification of genetic loci for susceptibility to osteoporosis is discussed in more detail below.

Linkage Analysis in Pedigrees

Classical linkage analysis in human pedigrees has been used widely and successfully for mapping single-disease genes. The principle of this approach is outlined in Figure 1, left panel. It involves genotyping family members for a number of polymorphic genetic markers, and then looking for an evidence of co-segregation of a particular marker with the disease phenotype under a pre-defined model of inheritance. Genetic markers located close to the disease-causing gene will co-segregate with the phenotype. Linkage is measured by lodscore and for Mendelian diseases is considered significant when the lodscore values exceed 3.0 (odds ratio of linkage against no linkage > 1000) and can be excluded when lodscore values are less than -2.0 . Suggestive linkage is defined when lodscore values are between 2.2-3.0. For complex diseases where linkage studies are generally performed using non-parametric analysis methods however, the significance thresholds are higher and a lodscore of above 3.6 must be obtained for significant linkage (16). Linkage studies are usually performed on a genome-wide basis using a fixed panel of microsatellite markers that are approximately 10 centimorgans apart although over recent years, panels of closely spaced single nucleotide polymorphism (SNP) markers have been developed that offer improved statistical power in performing genome wide scans for complex diseases (17). Genome wide scans using the classical linkage approach have been successfully used to map genetic loci for rare monogenic bone diseases such as osteoporosis-pseudoglioma syndrome and high bone mass trait (18;19). However this approach is less suitable for the study of complex diseases such as osteoporosis or complex traits such as BMD because of the difficulty in finding multigenerational informative families and unknown mode of inheritance.

Another linkage analysis method for mapping quantitative trait loci (QTLs) and complex disease genes has been developed to tackle the limitation of the classical linkage methods. The analysis is based on allele sharing in sib pairs (Figure 1, middle panel). Allele sharing methods involve testing whether affected relatives inherit a certain chromosomal region more often than expected under random Mendelian segregation. This method, in contrast to classical linkage studies, is not affected by locus heterogeneity and does not require construction of a disease model. Therefore, allele sharing method has been the most widely used approach for mapping complex disease genes such as osteoporosis. However, the success of this approach is dependent on the availability of a large number of sib-pairs to gain adequate statistical power to detect genes with modest effect. The first genome wide linkage search to identify quantitative trait loci (QTLs) that contribute to the genetic variations of BMD was performed by Devoto and colleagues in families with history of osteoporosis (20). The authors analysed the data using two different methods; the classical linkage analysis and allele sharing methods. While none of the QTLs identified by the classical linkage approach reached the statistical significance for linkage, several QTLs with suggestive or significant linkage were identified when the data were analysed using the allele sharing methods (Table 1) demonstrating the fact that classical linkage analysis is less suitable for mapping complex disease genes.

Another statistical method to perform genome wide linkage scans was developed by Almasy and Blangero (21), based on variance component linkage analysis. The advantage of this approach is that it allows estimating the contribution of possible covariates affecting the phenotype under investigation. When studying BMD, the contribution of environmental factors (such as calcium intake, physical activity, smoking and estrogen use) as well as other important confounding factors such as age, weight and body mass index can be included in the variance component analysis. The variance component method has been used by many investigators to perform genome wide scans to identify QTL that regulate BMD and other osteoporosis related phenotypes. Table 1 summarises the QTLs for BMD regulation identified by various genome wide linkage studies with significant or suggestive linkage.

Lee and colleagues have recently performed a meta-analysis of 11 previously published genome-wide scans in order to assess evidence for linkage of BMD across whole genome scan

studies (22). They analysed the data from ~3,000 families and found that the region on chromosome 16pter-16p12.3 has the greatest evidence of linkage. An interesting finding to emerge from this meta-analysis is the identification of two chromosomal regions (10p14-q11, 22q12-pter) with evidence for linkage that have not been detected by individual studies.

However, the authors used published linkage scores instead of whole genome data which reduces the statistical power of the study and the analysis did not take into account the ethnic differences in genetic loci for BMD regulation.

Genome wide scans for regulation of other osteoporosis-related phenotypes such as hip geometry and quantitative ultrasound properties of bone have been reported. Several loci for regulation of various aspects of femoral neck geometry were identified in a linkage study performed by Koller *et al.*(23). Wilson and colleagues identified two QTLs for quantitative ultrasound of the calcaneus on chromosome 2q33-37 and 4q12-21 in a large twin cohort (24). Sex-specific QTL for bone structure at the proximal femur have been also been reported (25). Several important observations have emerged from the linkage studies that have been performed in the field of osteoporosis genetics. It is now clear that most of the loci that regulate BMD do so in a site specific and gender specific manner although a few QTLs have been identified where there are effects in both genders and at BMD at more than one skeletal site. Another feature to emerge from studies that categorised patients by age group is that the loci regulating peak bone mass are probably different from those that regulating BMD in older people (26). This raises the possibility that genes which regulate BMD may differ from those that regulate bone loss, although in point of fact the evidence in favour of genetic effects on bone loss is very limited. Another important point is that few genome wide scans have actually identified loci that reach the threshold for genome wide significance and so far, only one gene for osteoporosis susceptibility has actually been identified so far by linkage and positional cloning and that was the bone morphogenetic protein 2 (*BMP2*) in the isolated population of Iceland (27).

Animal studies

Based on the assumption that key genes regulating BMD will be shared across species, linkage studies in model animals provide another approach to study the genetic basis of osteoporosis.

The principle of this approach is outlined in Figure 1, right panel. A cross of one inbred mouse strain with high BMD and another with low BMD is usually performed and followed by a brother-sister mating of the F1 animals to generate F2 strain of animals with varying levels of BMD due to segregation of the alleles that regulate BMD. A genome wide scan is performed in the F2 generation to identify QTLs that regulate BMD. The first genome search looking for QTLs for bone mass regulation in mice was performed by Klein and colleagues who identified 10 genetic loci that were linked to bone mass in female mice and four other loci linked to body weight (28). Subsequent genome searches by various investigators identified many other QTLs for BMD regulation which are distributed across the mouse chromosomes (29-31). Gene mapping studies in mice have identified QTLs for other bone phenotypes of relevance to osteoporosis including femoral cross-sectional area (32), trabecular bone volume and microarchitecture (33) and mechanical properties of mouse femur (34). Interestingly, QTLs regulating bone phenotypes in mice were found to be gender-specific (35;36) and skeletal-site specific (37;38) consistent with the observations reported in humans. Although many QTLs identified are specific to individual mouse strains, replication of some QTLs for BMD regulation has been reported between different strains suggesting that some of the genetic variants in these loci may be highly conserved. Furthermore, some QTLs for regulation of BMD identified in the mouse show synteny with QTLs identified in humans. An example is the QTL identified on mouse chromosome 4 near the marker D4Mit312 (Lodscore = 12.3) which is homologous to the QTL on the human chromosome 1p36 identified by various linkage studies (Table 1). Since most QTL mapping have been performed in inbred strains, fine mapping attempts to narrow down the QTL have been reported (39) but so far there has been limited success in identifying the causative gene. A notable exception was in the case of a mouse chromosome 11 QTL identified by Klein and colleagues. Here the investigators studied the expression profile for genes within the region using microarray technology and they found that the expression level of *Alox15* in DBA2 strain of mice (low BMD strain) was 20 fold more than the expression level in C57BL/6 mice (high BMD strain) suggesting that *Alox15* might act as a negative regulator of peak bone mass in mice (40). Consistent with this hypothesis, *Alox15* knockout mice had increased BMD. A recent study has also shown that polymorphisms in the

human *ALOX15* gene were associated with BMD in a study of postmenopausal Japanese women (41). The mechanism by which *Alox15* regulates BMD is still unclear but it has been postulated that it may be involved in osteoblast and adipocyte differentiation through activation of the PPAR gamma receptor. A recent study has also shown that the human chromosome Xp22 region, which is syntenic to a QTL for BMD regulation identified on mouse chromosome X, may also contain genes that regulate BMD in humans demonstrating the possibility of transferring loci for BMD regulation between the mouse and the human genomes (42). In summary, linkage studies in mice have identified many QTLs for BMD regulation but further studies will be required to identify the genes responsible and to investigate if these genes are also involved in the pathogenesis of osteoporosis in humans.

Association studies of candidate genes

The vast majority of information on the genes associated with osteoporosis susceptibility came from association studies in candidate genes. Association studies are based on comparing allele frequency for a polymorphism in or around a candidate gene of interest in a case and control group of individuals. Disease-associated alleles will be over-represented in affected individuals as compared with controls. Quantitative traits such as BMD can also be investigated by comparing the mean values of the trait in different genotype groups for the polymorphisms under study. The principle of the classical association study is outlined in Figure 2.

Recently, however, it has become possible to perform association studies on a genome wide basis by analyzing a large number of closely spaced single nucleotide polymorphisms (SNP) spread randomly across the genome (43). The rationale for these studies is that these SNPs will be in linkage disequilibrium with causal variants in genes that predispose to the disease under study. Genome wide association studies represent a major challenge in terms of statistical analysis, but the very high density of marker panels now available offers the prospect of identifying genes and genomic regions which contribute to complex diseases such as osteoporosis.

The most widely studied candidate genes for BMD regulation involved those with obvious function in bone physiology such as the Vitamin D receptor (*VDR*), the collagen type I alpha 1

(*COL1A1*) and the estrogen receptor alpha gene (*ESR1*). Other candidate genes were studied in relation to osteoporosis because of their involvement in rare bone disorders affecting BMD regulation such as the chloride channel (*CLCN7*). Candidate genes located in regions of suggestive or significant linkage for BMD regulation identified by genome wide scans have also been investigated for their association with osteoporosis-related phenotypes. The most widely studied candidate genes that have been implicated in the regulation of BMD and other osteoporosis-related phenotypes are discussed in more detail below.

Vitamin D Receptor

Vitamin D has important effects on bone and calcium metabolism through interaction with its nuclear receptor; the vitamin D receptor encoded by the *VDR* gene.

The *VDR* was the first candidate gene to be studied in relation to osteoporosis and several *VDR* polymorphisms have now been studied in relation to BMD and other bone related phenotypes such as bone loss, osteocalcin levels, and osteoporotic fracture (44;45). The most widely studied polymorphisms are: *BsmI* and *Apal* located in intron 8, and *TaqI* which is a conservative T→C change located in exon 9. Following the first report of an association between these polymorphisms and BMD by Morrison *et al* (44), a large number of studies were performed with inconsistent results. The majority of these studies have been summarised in two recent meta-analyses performed by Gong *et al* (46) and Thakkestian *et al* (47). The first study analysed the outcome of 75 studies published between 1994 and 1998 and concluded that there is a significant association between *VDR* polymorphisms and BMD, although the effect is small and positive association with *VDR* polymorphisms were more common in studies which included premenopausal women rather than postmenopausal women. Furthermore, they suggested that the association may have been missed in some studies because of small sample size and the effect of other confounding factors. Consistent with this suggestion, the relationship between *VDR* polymorphisms and BMD has been reported to be modified by environmental factors such as calcium and vitamin D intake (48). The second meta-analysis analysed studies published between 1994 and 2001 and included those which only genotyped the *BsmI* polymorphism in women (47). The study found an evidence for lower lumbar spine

BMD (2.4%) in “*BsmI*” BB compared to Bb/bb genotypes, but no evidence for an association with femoral neck BMD. Fracture has also been studied by various investigators in relation to the *BsmI* VDR polymorphism but results were inconsistent (45;49;50) .

Functional studies performed to assess the effect of the *BsmI*, *ApaI* and *TaqI* polymorphisms on VDR function and/or gene transcription have yielded inconsistent results. One study reported an evidence of haplotype-specific differences in gene transcription using reporter gene constructs prepared from the 3’ region of the VDR gene (44). Other studies have shown no differences in allele-specific transcription, mRNA stability, or ligand binding (51) in relation to the polymorphisms in the 3’ region of the VDR gene suggesting that these polymorphisms might be in linkage disequilibrium with other functional polymorphisms located in the VDR gene.

A functional polymorphism (recognised by the *FokI* restriction enzyme) in exon 2 of the VDR gene has been identified which introduces an alternative translational start site resulting in a shorter isoform of the VDR gene (52). Studies on the association between the *FokI* polymorphism and BMD yielded inconsistent result (52) and studies on the effect of this polymorphism on the VDR function and/or transcription level were inconclusive (53). Further studies are required to elucidate the effects of this polymorphism.

Another potentially functional polymorphism has been described in the VDR promoter at a binding site for the transcription factor Cdx-2 and found to be associated with BMD in Japanese subjects (54). It was also reported to influence DNA protein binding and to modulate gene expression in reporter assays (54).

Recently, a large scale study of the VDR gene in relation to osteoporosis related phenotype has been reported by Fang and colleagues (55). Participants of the Rotterdam study (n=6148) were genotyped for 15 haplotype-tagging SNPs selected after construction of linkage disequilibrium blocks across the VDR gene. The authors identified haplotype alleles in the promoter and the 3’ untranslated region (UTR) that were associated with increased risk of fracture. They observed 48% increase in fracture risk in the subgroup of individuals (16%) who had risk genotypes at both regions, and this was independent of bone mineral density. Functional analysis of VDR variants showed lower expression of a reporter construct with promoter risk haplotype and lower mRNA level of VDR expression constructs carrying 3'-UTR risk haplotype associated

with increased degradation of *VDR* mRNA. Therefore, carriers of risk haplotypes at both promoter and 3'UTR region have lower *VDR* mRNA levels attributed to the combined effect of decreased transcription and increased degradation of *VDR* mRNA. The authors suggested that lower *VDR* levels could affect the vitamin D signaling efficiency which might contribute to the increased fracture risk, although the mechanism by which these variants predispose to fracture is unclear. Since the observed increase in fracture risk associated with risk haplotypes was independent of BMD, a possibility of an effect on bone geometry might explain the observed increase in fracture risk; however, further studies will be required to confirm this hypothesis.

Collagen type I alpha 1

Collagen is the main structural protein of bone and is encoded by two separate genes; the *COL1A1* gene which encodes the alpha 1 chain of type I collagen and the *COL1A2* gene which encodes the alpha 2 chain of type 1 collagen. Both genes are important functional candidate genes for the genetic regulation of bone mass and osteoporosis since mutations in these genes account for the vast majority of osteogenesis imperfecta cases (56). Whilst mutations affecting the coding region of the type I collagen genes have been excluded as a common cause of osteoporosis (57), attention has focused on the possibility that more subtle polymorphisms affecting the regulatory region of the collagen genes might predispose to osteoporosis. Most attention has focused on a common polymorphism (G → T) located in intron 1 of the *COL1A1* gene which was found to alter a Sp1 transcription binding site (58). Grant and colleagues reported a strong association between this polymorphism and osteoporosis (58). Following this report, extensive studies have been performed on this polymorphism showing an association with BMD (58;59), and other osteoporosis-related phenotypes such as fracture risk (58;59), bone loss (60;61), bone geometry (62), bone mineralization (63) and bone quality(64). However, some studies reported no association between the *COL1A1* Sp1 polymorphism and BMD or fracture. Three meta-analyses of published studies investigating the Sp1 polymorphism have been performed and concluded that the “T” allele of this polymorphism is associated with reduced BMD at lumbar spine and femoral neck and with increased risk of vertebral fractures (64-66). Similar conclusions were reported in the large multi-centre

GENOMOS study (67) where the Sp1 polymorphism was genotyped in 20,786 individuals from several centres across Europe and significant associations with BMD and incident vertebral fractures were found. Interestingly, this study showed a recessive effect of the Sp1 polymorphism on BMD contrasting with a co-dominant effect reported in previous studies, although the association with vertebral fracture was mediated by a co-dominant effect as previously reported.

Functional studies on the *COL1A1* Sp1 polymorphism have shown evidence that the “T” allele is associated with increased binding affinity for Sp1 protein, three fold increase in the primary RNA transcripts, and increased production of type I alpha protein (64). Furthermore, biomechanical testing of bone samples showed reduced bone strength and a slight reduction of mineralisation of bone from samples with “T” allele (63).

Recently, two polymorphisms (-1997G/T and -1663delT) have been described in the promoter of *COL1A1* gene that are in linkage disequilibrium with the Sp1 polymorphism and were found to be associated with BMD in some (68) but not other studies (69-71). Functional analysis using reporter assays showed that these polymorphisms may impact *COL1A1* transcription (72). More recently, a large study which included the promoter and the Sp1 polymorphism showed a consistent association with haplotypes which had opposing effects on both hip and spine BMD. These were haplotype 2 (-1997G / -1663DelT / Sp1”T”), which was significantly associated with low BMD and haplotype 3 (-1997T / -1663InsT / Sp1”G”) which was significantly associated with high BMD (Stewart *et al*, 2006, *submitted*). These findings suggest that the association observed with Sp1 polymorphism previously reported by various studies may actually be driven by an extended haplotype spanning the promoter and intron 1 of *COL1A1* gene and the inconsistent results reported for the Sp1 polymorphism may be attributed to differences in the pattern of linkage disequilibrium between different populations.

Estrogen receptor alpha

In view of the fact that oestrogen deficiency following the menopause is a major risk factor for osteoporosis, the oestrogen receptor alpha encoded by the *ESR1* gene represent an important candidate gene for the genetic control of osteoporosis. Three main polymorphisms of the *ESR1*

gene have been investigated; a TA repeat polymorphism in promoter region and two SNPs located in the first intron and defined by the restriction enzymes *PvuII* and *XbaI*.

The TA repeat polymorphism was first reported to be associated with BMD by Sano *et al* (73) and the intron1 *PvuII* and *XbaI* polymorphisms were first shown to be associated with BMD in a different study of Japanese subjects (74). Subsequently, these polymorphisms have been investigated in relation to BMD (74-76) and other osteoporosis related phenotypes (77;78) with mixed results. Ioannidis and colleagues analysed 22 studies published between 1996 and 2001 in a meta-analysis which showed evidence for an association between *XbaI* polymorphism and both BMD and fracture (79). They found that “XX” homozygotes were associated with higher BMD values and reduced risk of fractures compared to other genotype groups. A more recent and large scale prospective meta-analysis of data from 18,917 individuals from the GENOMOS study showed that “XX” homozygotes were associated with reduced fracture risk confirming the observation from the first meta-analysis (80). However, no association with BMD was observed in this study suggesting that *ESR1* polymorphisms might influence fracture risk by mechanisms that are independent of BMD such as bone quality. Consistent with this finding, a recent study have shown a significant association of *ESR1* polymorphisms with ultrasound properties of bone and rates of postmenopausal bone loss in a large cohort of ~3000 women (81).

The data assembled so far indicate that allelic variation at the *ESR1* gene contributes to the genetic regulation of osteoporosis. Although there is an evidence from reporter gene assays to suggest that the *PvuII* polymorphism creates a functional binding site for the transcription factor B-Myb (82), the impact of this polymorphism on *ESR1* transcription has not been determined and further studies are required to elucidate the mechanisms by which *ESR1* alleles regulate bone-phenotypes.

Transforming Growth Factor beta 1

The *TGFβ1* gene has been extensively studied as a potential regulator of susceptibility to osteoporosis partly because it is particularly abundant in bone and has been shown to have effects on both osteoblast and osteoclast function *in vitro* (83). One of the earliest studies was that of Langdahl and colleagues who identified a polymorphism within intron 4 of the *TGFβ1*

that was associated with severe osteoporosis (84). Subsequent work by the same group evaluated the relationship between several polymorphisms in *TGFβ1* and osteoporosis in a case control study and identified an association between a polymorphism located in the fifth intron and BMD (85). Other research has focused on polymorphisms in the promoter and first exon of *TGFβ1* in relation to BMD (86-88). A protein coding polymorphism causing a leucine to proline substitution in the signal peptide region of *TGFβ1* has been found to be associated with BMD and with circulating *TGFβ1* in some populations (86;88), although the mechanisms by which this polymorphism regulates BMD is unclear. There have been many studies of *TGFβ1* alleles in relation to BMD and other osteoporosis genotypes but most have been of limited sample size and as a consequence of this, somewhat conflicting results have been reported. Definitive evidence that genetic variation in *TGFβ1* can regulate bone mass in humans comes from the observation that Camurati-Engelmann disease (a rare bone dysplasia characterized by osteosclerosis affecting the diaphysis of long bones) is caused by mutations in *TGFβ1* (89;90). These mutations activate TGFβ1 signaling by inhibiting binding of the mature TGFβ1 peptide to the inhibitory latency associated peptide (91).

Lipoprotein Receptor Related protein 5 (LRP5)

Various mutations in the *LRP5* gene were recently found to be responsible for two rare bone disorders; osteoporosis-pseudoglioma syndrome (OPS, a disorder characterised by juvenile onset osteoporosis and visual loss) and autosomal dominant inheritance of high bone mass (HBM) (92-94). Inactivating mutations of *LRP5* are responsible for OPS, whereas gain-of function mutations are responsible for the HBM syndrome. The *LRP5* gene encodes a transmembrane receptor, which is involved in Wnt signaling (94) and several polymorphisms of the *LRP5* gene have now been investigated in relation to BMD. Ferrari and colleagues analysed several polymorphisms of the *LRP5* gene in relation to BMD and identified significant association between G2047A polymorphism and lumbar spine BMD, but the association was most significant in men (95). However, subsequent studies have reported association between BMD and various haplotypes defined by polymorphisms in the *LRP5* in both men and women (96;97).

Current evidence suggest that *LRP5* pathway regulates bone mass mainly by affecting bone formation, reflected by the fact that individuals with activating mutations have increased biochemical markers of bone formation, but no disturbance in bone resorption (94). Consistent with this suggestion, heterozygous *LRP5* knockout mice have decreased trabecular bone volume density (98) and mice with high bone mass *LRP5* G171V mutation have increased bone cross-sectional area and thickness (99). . Functional analysis of the high bone mass associated mutations of *LRP5* has shown that they probably cause activation of beta-catenin signaling by inhibiting interactions between LRP5 and the inhibitor of Wnt signalling Dkk1. An initial study by Boyden and colleagues (94) showed that the G171V mutation did not result in constitutive activation of LRP5 signaling *in vitro* but that it impaired Dkk1 mediated inhibition of Wnt stimulated LRP5 signaling. Another study reached the same conclusion in showing that several HBM-associated mutants (G171V, G171R, A214T, A214V, A242T, T253I and D111Y) were resistant to Dkk1 inhibition compared with wild type LRP5 and had lower affinity for Dkk1 binding (100). Although the mechanisms by which rare *LRP5* mutations affect bone turnover seems reasonably well worked out, further studies will be required to define the mechanism by which the more subtle polymorphisms in *LRP5* affect bone mass.

Core-Binding Factor A 1 (CBFA1)

The *CBFA1* gene (also known as *RUNX2*) plays an essential role in regulating osteoblast differentiation since mice which are deficient in this transcription factor have complete absence of bone (101;102), whereas mice with haploinsufficiency of *CBFA1* phenocopy the human syndrome of cleidocranial dysplasia (CCD), a skeletal disorder characterized by short stature, hypoplasia or aplasia of the clavicles, patent fontanelles, supernumerary teeth and other defects in skeletal patterning and growth (102). The human syndrome of CCD is caused by various missense, nonsense and frameshift mutations of *CBFA1* (103). Various polymorphisms have been identified in *CBFA1* and some of these have been associated with bone mass in population based studies (104-106). The best functional candidate polymorphism lie within the *CBFA1* promoter or within polyalanine and polyglutamine repeats in exon 1 (104). The polyalanine and polyglutamine repeats are of special interest since they lie within one of the transactivation

domains of CBFA1. Various polymorphic variations have been identified in this region including a 18bp deletion which results in a polyalanine repeat of 11 residues (11 ala) compared with the more common repeat of 17 residues (17 ala). Various rare length variants within the polyglutamine repeat have also been identified resulting in stretches of between 15 and 30 repeats. The strongest association with BMD has been observed with an anonymous polymorphism in the Ala repeat region (105;106), although it is thought that this might be due to linkage disequilibrium with polymorphisms in the promoter which have been shown to affect *CBFA1* transcription in reporter assays (104). It is currently unclear whether the length variants in the polyalanine and polyglutamine tracts have functional importance, but this is an area of ongoing investigation.

Tumor Necrosis Factor Receptor Superfamily 1B (TNFRSF1B)

The *TNFRSF1B* (also known as *TNFR2*) mediates the effects of TNF which have an important role in regulating bone turnover. Specifically, TNF- α induced activation of the *TNFRSF1B* receptor has been found to suppress osteoclastogenesis *in vitro*, contrasting with its effects on the *TNFRSF1A* receptor which results in enhanced osteoclastogenesis (107). The *TNFRSF1B* gene is located on chromosome 1p36 region which was found to be linked to BMD in three independent studies, rendering it both a positional and functional candidate for the regulation of BMD (20;108;109). Polymorphisms in the 3'UTR of *TNFRSF1B* have been reported to be associated with spine BMD in a small population based study of American population (110). However, a larger study in Scottish women showed an association with femoral neck but not lumbar spine BMD (111) consistent with the linkage findings identified by genome wide linkage scan (20). Since these polymorphisms are located in the 3'UTR, it has been postulated that these polymorphisms might influence *TNFRSF1B* mRNA level by affecting the mRNA structure and stability.

CLCN7 and TCIRG1

Mutations in genes encoding chloride channel 7 (*CLCN7*) and osteoclast specific proton pump (*TCIRG1*) have been found to cause some forms of osteopetrosis (112), a disease characterised by increased BMD due to impaired osteoclast function. Both genes are highly expressed in

osteoclast and play a significant role in acidification of the lacunae during bone resorption. Polymorphisms in the *CLCN7* and *TCIRG1* have been reported to be associated with BMD (113;114), however, further studies in different population will be required to confirm these findings.

Other candidate genes

Polymorphisms of many other candidate genes have been studied in relation to BMD and other osteoporosis-related genotypes (reviewed by Liu *et al* (115)). Constraints of space limit full discussion of these genes, which in general have been investigated in populations with a limited sample size. Further studies will be required to confirm their candidacy as genetic regulators of bone mass.

Conclusion

Many advances have been made in understanding the mechanisms by which genetic factors regulate susceptibility to osteoporosis over the past 10 years. It has become clear from studies in man and experimental animals that different genes regulate BMD at different skeletal sites and in men and women. Linkage studies have identified several chromosomal regions that regulate BMD but only a few causative genes have been discovered so far using this approach. In contrast, significant advances have been made in identifying the genes that cause monogenic bone diseases and polymorphic variation in some of these genes has been found to contribute to the genetic regulation of BMD in the normal population. Other genes which have been investigated as possible candidates for susceptibility to osteoporosis such as vitamin D because of their role in bone biology have yielded mixed results. Many candidate gene association studies have been underpowered and meta-analysis has been used to try to confirm or refute potential association and gain a better estimate of their true effect size in the population. Most of the genetic variants which confer susceptibility to osteoporosis remain to be discovered and it is likely that new techniques such as whole –genome association will provide new insights into the genetic determinants of osteoporosis and will help to identify genes of modest effect size. From a clinical standpoint, genetic variants that are found to predispose to osteoporosis will advance

our understanding of the pathophysiology of the disease and could be developed as diagnostic genetic tests or form molecular target for design of new drugs for the prevention and treatment of osteoporosis and other bone diseases.

Figure 1. Genetic approaches used in the identification of osteoporosis susceptibility genes.

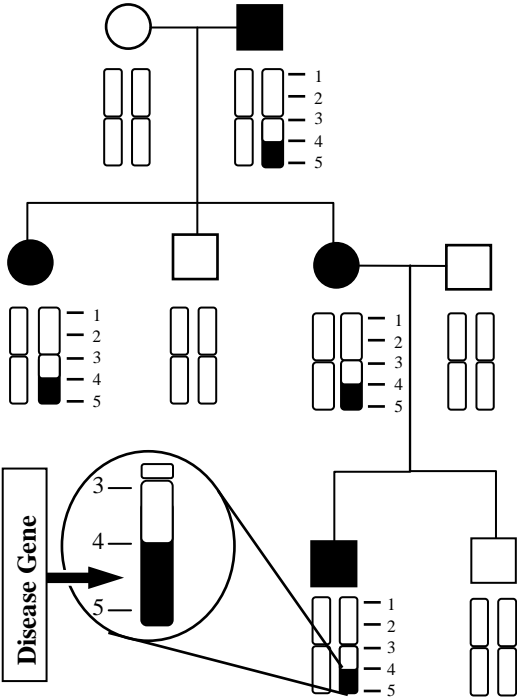
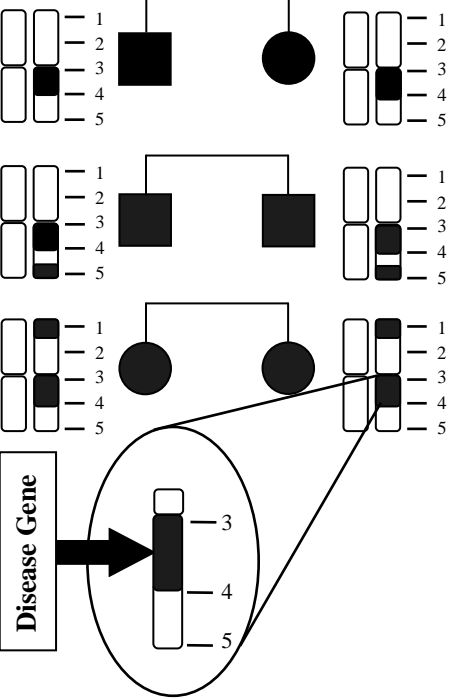
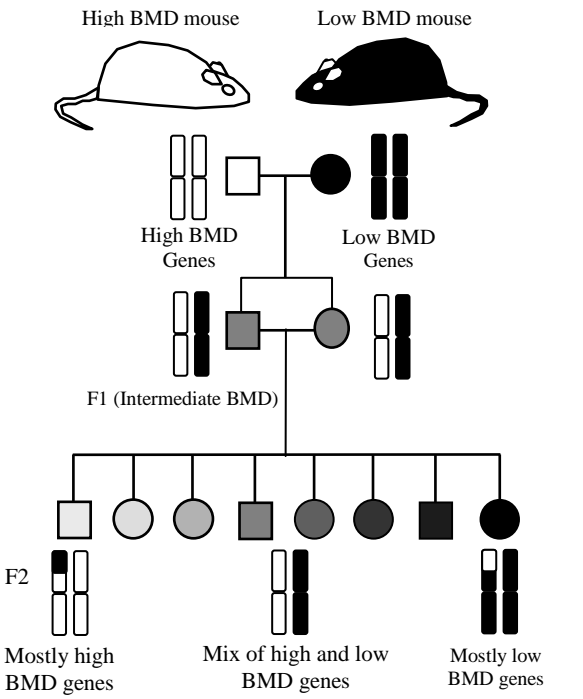
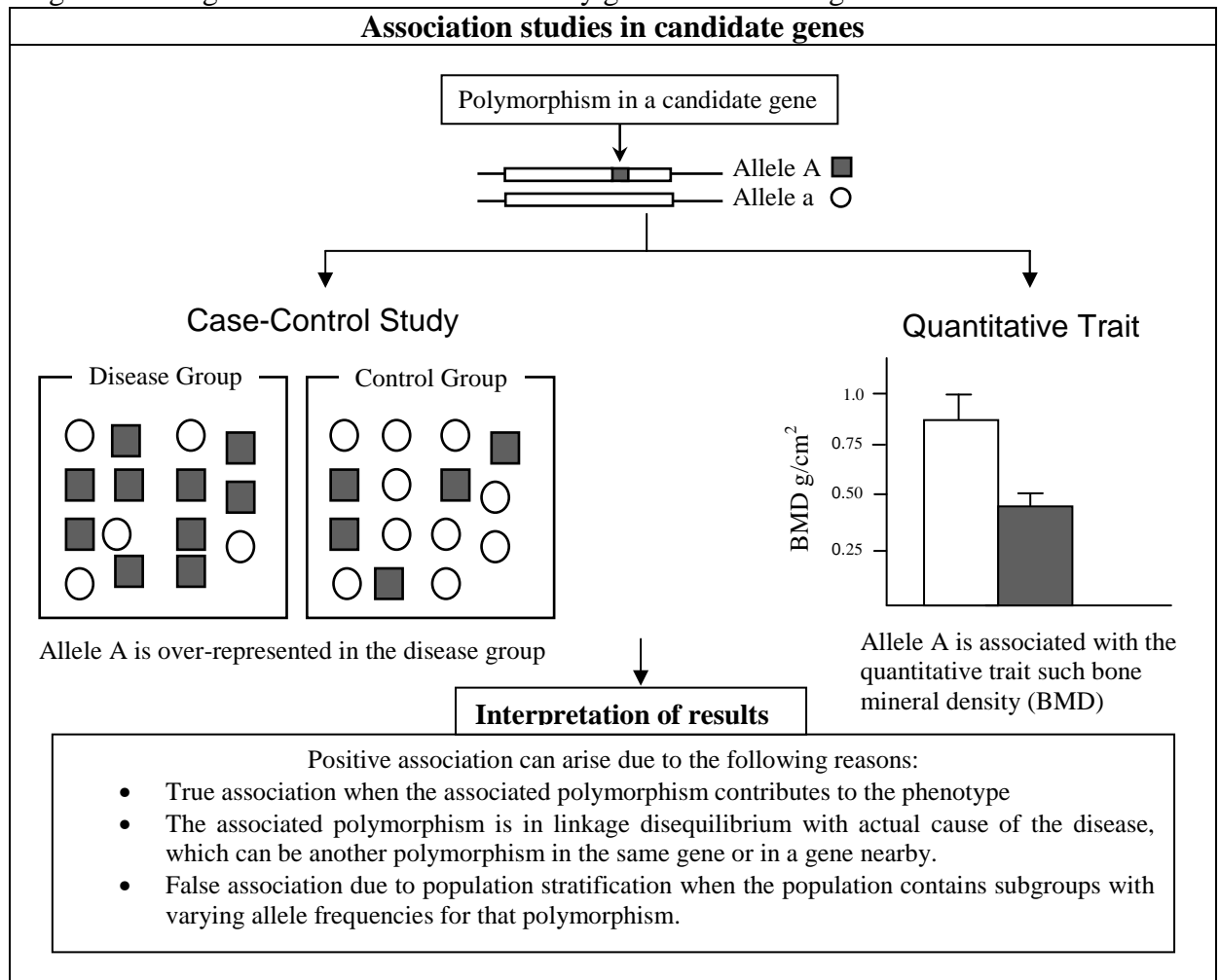
Classical linkage analysis	Allele-sharing in sib-pairs	Linkage analysis in animal models
		
<p>Principle: Look for co-segregation of a polymorphic marker with the disease in pedigrees. In the above example markers 4 and 5 are segregating with the disease status indicating that the disease gene is located between these two markers.</p> <p>Advantage: Suitable for mapping single-gene or oligogenic disorders.</p> <p>Disadvantages: requires large multigenerational pedigrees and defined model of inheritance.</p>	<p>Principle: Test whether affected relatives share certain chromosomal region more often than expected by random segregation. In the above example the region between marker 3 and 4 is shared between all affected sib-pairs indicating that the disease gene may be located between these two markers</p> <p>Advantage: more suitable for mapping complex disease genes when mode of inheritance can not be defined.</p> <p>Disadvantages: large number of sib-pairs is required.</p>	<p>Principle: Involve crosses between two different strains of animals. In the above example a mouse strain with low BMD is crossed with a high BMD strain and genetic loci can be identified by analysis of F2 generation looking for co-segregation of genetic markers with BMD loci.</p> <p>Advantage: large number of animals can be obtained, breeding programme can be controlled</p> <p>Disadvantages: Loci identified may be species-specific, requires animal model for the phenotype under investigation.</p>

Table 1. Major quantitative trait loci for BMD identified by linkage analysis in humans

Study	Study Design	Statistical approach	Chromosome	Lod score	Phenotype	Gender
Devoto <i>et al</i> (1998) (20)	History of osteoporosis	Allele-sharing	1p36	2.29	FN-BMD	M&F
			2p23-24	2.25	LS-BMD	M&F
			4qter	2.28	FN-BMD	M&F
Niu <i>et al</i> (1999) (116)	Healthy sib-pair	Allele-sharing	2p21-24	2.15	Forearm-BMD	M&F
Koller <i>et al</i> (2000) (117)	Healthy sib-pair	Allele-sharing	1q21-23	3.11	LS-BMD	F
Karasik <i>et al</i> (2002) (118)	Normal Population	Variance component	21q22.2	2.39	TR-BMD	M&F
			21qter	3.14	TR-BMD	M&F
Deng <i>et al</i> (2002) (119)	Proband with low BMD	Variance component	4q31-32	3.08	Spine BMD	M&F
			10q26	2.29	FN- BMD	M&F
			13q33-34	2.43	LS- BMD	M&F
Karasik <i>et al</i> (2003) (120)	Normal Population	Variance component	9q22-31	2.71	FN-BMD	F
			12q23-24	3.00	TR-BMD	F
			14q31	2.48	LS-BMD	F
Kammerer <i>et al</i> (2003) (121)	Normal Population	Variance component	2p25	3.98	FN-BMD	M
			4p15	4.33	Forearm BMD	M & F
			6q27	2.27	TR-BMD	M & F
			12q24	2.24	Forearm BMD	M & F
			13q14	3.46	TR-BMD	M
			13q14	2.51	FN-BMD	M
Wilson <i>et al</i> (2003) (109)	Dizygotic female twins	Allele sharing	1p36	2.38	Whole body BMD	F
			3p21	2.72	LS-BMD	F
Styrkarsdottir <i>et al</i> (2003)(27)	History of osteoporosis	Allele-sharing	20p12	3.18	FN-BMD	M & F
			20p12	2.89	LS-BMD	M & F
Shen <i>et al</i> (2004) (122)	Normal population + probands with low BMD	Variance component	11q23	3.13	LS-BMD	M & F
			Xq27	4.30	Forearm BMD	M & F
			Xq27	2.57	FN-BMD	M & F
Ralston <i>et al</i> (2005) (26)	History of osteoporosis	Variance component	3q25	2.43	FN-BMD	M
			4q25	2.22	FN-BMD	M
			4q25	2.55	FN-BMD	F
			7p14	2.28	FN-BMD	M
			10q21	4.2	FN-BMD	M
			16p13	2.52	FN-BMD	M
			16q23	2.28	LS-BMD	F
			18p11	2.83	LS-BMD	F
			20q13	3.2	LS-BMD	F
Peacock <i>et al</i> (2005) (123)	Normal population	Allele sharing	1q	3.13	LS-BMD	M
			2p	4.4	LS-BMD	M & F
			2q	2.99	FN-BMD	M
			14p	4.6	LS-BMD	M

Abbreviations: FN= femoral neck, LS= Lumbar spine, TR=trochanter, BMD= bone mineral density, M=Males, F= Females

Figure 2. Using association studies to identify genes for BMD regulation.



Reference List

- (1) Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE *et al.* Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* 1995; 332(12):767-773.
- (2) Soroko SB, Barret-Connor E, Edelstein SL, Kritz-Silverstein D. Family history of osteoporosis and bone mineral density at the axial skeleton: The Rancho Bernardo study. *J Bone Miner Res* 1994; 9:761-769.
- (3) Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults: a twin study. *J Clin Invest* 1987; 80:706-710.
- (4) Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CC. Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. *J Bone Miner Res* 1991; 6:561-567.
- (5) Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. *J Bone Miner Res* 1995; 12:2017-2022.
- (6) Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. *J Bone Miner Res* 1993; 8:1-9.
- (7) Torgerson DJ, Campbell MK, Thomas RE, Reid DM. Prediction of perimenopausal fractures by bone mineral density and other risk factors. *J Bone Miner Res* 1996; 11(2):293-297.
- (8) Arden NK, Baker J, Hogg C, Baan K, Spector TD. The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. *J Bone Miner Res* 1996; 11:530-534.
- (9) Arden NK, Spector TD. Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *J Bone Miner Res* 1997; 12(12):2076-2081.
- (10) Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV *et al.* Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. *J Bone Miner Res* 2001; 16(2):371-378.
- (11) Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M, Rose RJ. Common genetic influences on BMI and age at menarche. *Human Biology* 1995; 67(5):739-753.
- (12) Kannus P, Palvanen M, Kaprio J, Parkkari J, Koskenvuo M. Genetic factors and osteoporotic fractures in elderly people: prospective 25 year follow up of a nationwide cohort of elderly Finnish twins. *Br Med J* 1999; 319(7221):1334-1337.
- (13) Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM *et al.* Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. *J Bone Miner Res* 2000; 15(7):1243-1252.
- (14) Andrew T, Antoniadou L, Scurrah KJ, MacGregor AJ, Spector TD. Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. *J Bone Miner Res* 2005; 20(1):67-74.
- (15) Michaelsson K, Melhus H, Ferm H, Ahlbom A, Pedersen NL. Genetic liability to fractures in the elderly. *Arch Intern Med* 2005; 165(16):1825-1830.
- (16) Nyholt DR. All LODs are not created equal. *Am J Hum Genet* 2000; 67(2):282-288.

- (17) Sawcer SJ, Maranian M, Singlehurst S, Yeo T, Compston A, Daly MJ *et al.* Enhancing linkage analysis of complex disorders: an evaluation of high-density genotyping. *Hum Mol Genet* 2004; 13(17):1943-1949.
- (18) Gong Y, Vikkula M, Boon L, Lui J, Beighton P, Ramesar R *et al.* Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12-13. *Am J Hum Genet* 1996; 59(1):146-151.
- (19) Johnson ML, Gong G, Kimberling W, Recker S, Kimmel DB, Recker RR. Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). *Am J Hum Genet* 1997; 60:1326-1332.
- (20) Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP *et al.* First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4q. *Eur J Hum Genet* 1998; 6:151-157.
- (21) Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998; 62(5):1198-1211.
- (22) Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. Meta-analysis of genome-wide linkage studies for bone mineral density. *J Hum Genet* 2006.
- (23) Koller DL, Liu G, Econs MJ, Hui SL, Morin PA, Joslyn G *et al.* Genome screen for quantitative trait loci underlying normal variation in femoral structure. *J Bone Miner Res* 2001; 16(6):985-991.
- (24) Wilson SG, Reed PW, Andrew T, Barber MJ, Lindersson M, Langdown M *et al.* A genome-screen of a large twin cohort reveals linkage for quantitative ultrasound of the calcaneus to 2q33-37 and 4q12-21. *J Bone Miner Res* 2004; 19(2):270-277.
- (25) Peacock M, Koller DL, Lai D, Hui S, Foroud T, Econs MJ. Sex-specific quantitative trait loci contribute to normal variation in bone structure at the proximal femur in men. *Bone* 2005; 37(4):467-473.
- (26) Ralston SH, Galwey N, MacKay I, Albagha OM, Cardon L, Compston JE *et al.* Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. *Hum Mol Genet* 2005; 14(7):943-951.
- (27) Styrkarsdottir U, Cazier JB, Kong A, Rolfsson O, Larsen H, Bjarnadottir E *et al.* Linkage of Osteoporosis to Chromosome 20p12 and Association to BMP2. *PLoS Biol* 2003; 1(3):E69.
- (28) Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES. Quantitative trait loci affecting peak bone mineral density in mice [see comments]. *J Bone Miner Res* 1998; 13(11):1648-1656.
- (29) Shimizu M, Higuchi K, Bennett B, Xia C, Tsuboyama T, Kasai S *et al.* Identification of peak bone mass QTL in a spontaneously osteoporotic mouse strain. *Mamm Genome* 1999; 10:81-87.
- (30) Beamer WG, Shultz KL, Churchill GA, Frankel WN, Baylink DJ, Rosen CJ *et al.* Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. *Mamm Genome* 1999; 10(11):1043-1049.
- (31) Benes H, Weinstein RS, Zheng W, Thaden JJ, Jilka RL, Manolagas SC *et al.* Chromosomal mapping of osteopenia-associated quantitative trait loci using closely related mouse strains. *J Bone Miner Res* 2000; 15(4):626-633.
- (32) Klein RF, Turner RJ, Skinner LD, Vartanian KA, Serang M, Carlos AS *et al.* Mapping quantitative trait loci that influence femoral cross-sectional area in mice. *J Bone Miner Res* 2002; 17(10):1752-1760.
- (33) Bouxsein ML, Uchiyama T, Rosen CJ, Shultz KL, Donahue LR, Turner CH *et al.* Mapping quantitative trait loci for vertebral trabecular bone volume fraction and microarchitecture in mice. *J Bone Miner Res* 2004; 19(4):587-599.
- (34) Volkman SK, Galecki AT, Burke DT, Miller RA, Goldstein SA. Quantitative trait loci that modulate femoral mechanical properties in a genetically heterogeneous mouse population. *J Bone Miner Res* 2004; 19(9):1497-1505.

- (35) Orwoll ES, Belknap JK, Klein RF. Gender specificity in the genetic determinants of peak bone mass. *J Bone Miner Res* 2001; 16(11):1962-1971.
- (36) Turner CH, Sun Q, Schrieffer J, Pitner N, Price R, Bouxsein ML *et al.* Congenic mice reveal sex-specific genetic regulation of femoral structure and strength. *Calcif Tissue Int* 2003; 73(3):297-303.
- (37) Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR *et al.* Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. *J Bone Miner Res* 2001; 16(7):1195-1206.
- (38) Masinde GL, Li X, Gu W, Wergedal J, Mohan S, Baylink DJ. Quantitative trait loci for bone density in mice: the genes determining total skeletal density and femur density show little overlap in F2 mice. *Calcif Tissue Int* 2002; 71(5):421-428.
- (39) Klein OF, Carlos AS, Vartanian KA, Chambers VK, Turner EJ, Phillips TJ *et al.* Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. *J Bone Miner Res* 2001; 16(11):1953-1961.
- (40) Klein RF, Allard J, Avnur Z, Nikolcheva T, Rotstein D, Carlos AS *et al.* Regulation of bone mass in mice by the lipoxxygenase gene *Alox15*. *Science* 2004; 303(5655):229-232.
- (41) Urano T, Shiraki M, Fujita M, Hosoi T, Orimo H, Ouchi Y *et al.* Association of a single nucleotide polymorphism in the lipoxxygenase *ALOX15* 5'-flanking region (-5229G/A) with bone mineral density. *J Bone Miner Metab* 2005; 23(3):226-230.
- (42) Parsons CA, Mroczkowski HJ, McGuigan FE, Albagha OM, Manolagas S, Reid DM *et al.* Interspecies syntenic mapping identifies a quantitative trait locus for bone mineral density on human chromosome Xp22. *Hum Mol Genet* 2005; 14(21):3141-3148.
- (43) Cardon LR, Abecasis GR. Using haplotype blocks to map human complex trait loci. *Trends Genet* 2003; 19(3):135-140.
- (44) Morrison NA, Qi JC, Tokita A, Kelly P, Crofts L, Nguyen TV *et al.* Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; 367:284-287.
- (45) Uitterlinden AG, Weel AE, Burger H, Fang Y, van Duijn CM, Hofman A *et al.* Interaction between the vitamin D receptor gene and collagen type I α 1 gene in susceptibility for fracture. *J Bone Miner Res* 2001; 16(2):379-385.
- (46) Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW *et al.* The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporosis Int* 1999; 9(1):55-64.
- (47) Thakkestian A, D'Este C, Eisman J, Nguyen T, Attia J. Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *J Bone Miner Res* 2004; 19(3):419-428.
- (48) Graafmans WC, Lips P, Ooms ME, van Leeuwen JPTM, Pols HAP, Uitterlinden AG. The effect of Vitamin D supplementation on the Bone Mineral Density of the Femoral Neck is associated with Vitamin D receptor genotype. *J Bone Miner Res* 1997; 12(8):1241-1245.
- (49) Feskanich D, Hunter DJ, Willett WC, Hankinson SE, Hollis BW, Hough *et al.* Vitamin D receptor genotype and the risk of bone fractures in women. *Epidemiology* 1998; 9(5):535-539.
- (50) Ensrud KE, Stone K, Cauley JA, White C, Zmuda JM, Nguyen TV *et al.* Vitamin D receptor gene polymorphisms and the risk of fractures in older women. For the Study of Osteoporotic Fractures Research Group. *J Bone Miner Res* 1999; 14(10):1637-1645.
- (51) Gross C, Musiol IM, Eccleshall TR, Malloy PJ, Feldman D. Vitamin D receptor gene polymorphisms: analysis of ligand binding and hormone responsiveness in cultured skin fibroblasts. *Biochem Biophys Res Commun* 1998; 242(3):467-473.

- (52) Arai H, Miyamoto K-I, Taketani Y, Yamamoto H, Iemori Y, Morita K *et al.* A vitamin D receptor gene polymorphism in the translation initiation codon: Effect on protein activity and relation to bone mineral density in Japanese women. *J Bone Miner Res* 1997; 12:915-921.
- (53) Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY, Feldman D. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *J Bone Miner Res* 1998; 13(11):1691-1699.
- (54) Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K *et al.* The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *J Bone Miner Res* 2001; 16(7):1256-1264.
- (55) Fang Y, van Meurs JB, d'Alesio A, Jhamai M, Zhao H, Rivadeneira F *et al.* Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet* 2005; 77(5):807-823.
- (56) Rowe DW. Osteogenesis Imperfecta. In: Heersche JNM, Kanis JA, editors. *Bone and Mineral Research*. Amsterdam: Elsevier, 1991: 209-241.
- (57) Spotila LD, Colige A, Sereda L, Constantinou-Deltas CD, Whyte MP, Riggs BL *et al.* Mutation analysis of coding sequences for type I procollagen in individuals with low bone density. *J Bone Miner Res* 1994; 9:923-932.
- (58) Grant SFA, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 site in the collagen type I alpha 1 gene. *Nat Genet* 1996; 14:203-205.
- (59) Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA *et al.* Relation of alleles of the collagen type I α 1 gene to bone density and risk of osteoporotic fractures in postmenopausal women. *N Engl J Med* 1998; 338:1016-1022.
- (60) MacDonald HM, McGuigan FA, New SA, Campbell MK, Golden MH, Ralston SH *et al.* COL1A1 Sp1 polymorphism predicts perimenopausal and early postmenopausal spinal bone loss. *J Bone Miner Res* 2001; 16(9):1634-1641.
- (61) Harris SS, Patel MS, Cole DE, Dawson-Hughes B. Associations of the collagen type Ialpha1 Sp1 polymorphism with five- year rates of bone loss in older adults. *Calcif Tissue Int* 2000; 66(4):268-271.
- (62) Qureshi AM, McGuigan FEA, Seymour DG, Hutchison JD, Reid DM, Ralston SH. Association between COL1A1 Sp1 alleles and femoral neck geometry. *Calcif Tissue Int* 2001; 69(2):67-72.
- (63) Stewart TL, Roschger P, Misof BM, Mann V, Fratzl P, Klaushofer K *et al.* Association of COL1A1 Sp1 alleles with defective bone nodule formation in vitro and abnormal bone mineralization in vivo. *Calcif Tissue Int* 2005; 77(2):113-118.
- (64) Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP *et al.* A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *J Clin Invest* 2001; 107(7):899-907.
- (65) Mann V, Ralston SH. Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. *Bone* 2003; 32(6):711-717.
- (66) Efstathiadou Z, Tsatsoulis A, Ioannidis JP. Association of collagen Ialpha 1 Sp1 polymorphism with the risk of prevalent fractures: a meta-analysis. *J Bone Miner Res* 2001; 16(9):1586-1592.
- (67) Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P *et al.* Large-Scale Evidence for the Effect of the COL1A1 Sp1 Polymorphism on Osteoporosis Outcomes: The GENOMOS Study. *PLoS Med* 2006; 3(4):e90.

- (68) Garcia-Giralt N, Nogues X, Enjuanes A, Puig J, Mellibovsky L, Bay-Jensen A *et al.* Two new single nucleotide polymorphisms in the COL1A1 upstream regulatory region and their relationship with bone mineral density. *J Bone Miner Res* 2002; 17(3):384-393.
- (69) Zhang YY, Lei SF, Mo XY, Wang YB, Li MX, Deng HW. The -1997 G/T polymorphism in the COL1A1 upstream regulatory region is associated with hip bone mineral density (BMD) in Chinese nuclear families. *Calcif Tissue Int* 2005; 76(2):107-112.
- (70) Yamada Y, Ando F, Niino N, Shimokata H. Association of a -1997G-->T polymorphism of the collagen Ialpha1 gene with bone mineral density in postmenopausal Japanese women. *Hum Biol* 2005; 77(1):27-36.
- (71) Liu PY, Lu Y, Long JR, Xu FH, Shen H, Recker RR *et al.* Common variants at the PCOL2 and Sp1 binding sites of the COL1A1 gene and their interactive effect influence bone mineral density in Caucasians. *J Med Genet* 2004; 41(10):752-757.
- (72) Garcia-Giralt N, Enjuanes A, Bustamante M, Mellibovsky L, Nogues X, Carreras R *et al.* In vitro functional assay of alleles and haplotypes of two COL1A1-promoter SNPs. *Bone* 2005; 36(5):902-908.
- (73) Sano M, Inoue S, Hosoi T, Ouchi Y, Emi M, Shiraki M *et al.* Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. *Biochemical & Biophysical Research Communications* 1995; 217(1):378-383.
- (74) Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphisms of the estrogen receptor gene in post-menopausal women. *J Bone Miner Res* 1996; 11:306-311.
- (75) Willing M, Sowers M, Aron D, Clark MK, Burns T, Bunten C *et al.* Bone mineral density and its change in white women: estrogen and vitamin D receptor genotypes and their interaction. *J Bone Miner Res* 1998; 13(4):695-705.
- (76) Albagha OM, McGuigan FE, Reid DM, Ralston SH. Estrogen receptor alpha gene polymorphisms and bone mineral density: haplotype analysis in women from the United Kingdom. *J Bone Miner Res* 2001; 16(1):128-134.
- (77) Salmen T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, Saarikoski S *et al.* Early postmenopausal bone loss is associated with PvuII estrogen receptor gene polymorphism in Finnish women: effect of hormone replacement therapy. *J Bone Miner Res* 2000; 15(2):315-321.
- (78) van Meurs JB, Schuit SC, Weel AE, van der KM, Bergink AP, Arp PP *et al.* Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet* 2003; 12(14):1745-1754.
- (79) Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L *et al.* Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis. *J Bone Miner Res* 2002; 17(11):2048-2060.
- (80) Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB *et al.* Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. *JAMA* 2004; 292(17):2105-2114.
- (81) Albagha OM, Pettersson U, Stewart A, McGuigan FE, MacDonald HM, Reid DM *et al.* Association of oestrogen receptor alpha gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone. *J Med Genet* 2005; 42(3):240-246.
- (82) Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA *et al.* Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation* 2002; 105(16):1879-1882.
- (83) Massague J, Chen YG. Controlling TGF-beta signaling. *Genes Dev* 2000; 14(6):627-644.
- (84) Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF. A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal

women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. *Bone* 1997; 20(3):289-294.

- (85) Langdahl BL, Carstens M, Stenkjaer L, Eriksen EF. Polymorphisms in the transforming growth factor beta 1 gene and osteoporosis. *Bone* 2003; 32(3):297-310.
- (86) Yamada Y, Hosoi T, Makimoto F, Tanaka H, Seino Y, Ikeda K. Transforming growth factor beta-1 gene polymorphism and bone mineral density in Japanese adolescents. *Am J Med* 1999; 106(4):477-479.
- (87) Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R, Pfeilschifter J. Association of transforming growth factor-beta1 (TGFbeta1) T29 --> C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta1 in a population-based sample of postmenopausal german women. *Calcif Tissue Int* 2001; 69(6):315-320.
- (88) Yamada Y, Miyauchi A, Takagi Y, Tanaka M, Mizuno M, Harada A. Association of the C-509-->T polymorphism, alone or in combination with the T869-->C polymorphism, of the transforming growth factor-beta1 gene with bone mineral density and genetic susceptibility to osteoporosis in Japanese women. *J Mol Med* 2001; 79(2-3):149-156.
- (89) Kinoshita A, Saito T, Tomita H, Makita Y, Yoshida K, Ghadami M *et al.* Domain-specific mutations in TGFBI result in Camurati-Engelmann disease. *Nat Genet* 2000; 26(1):19-20.
- (90) Janssens K, Gershoni-Baruch R, Guanabens N, Migone N, Ralston S, Bonduelle M *et al.* Mutations in the gene encoding the latency-associated peptide of TGF- beta 1 cause Camurati-Engelmann disease. *Nat Genet* 2000; 26(3):273-275.
- (91) Janssens K, ten Dijke P, Ralston SH, Bergmann C, Van Hul W. Transforming growth factor-beta 1 mutations in Camurati-Engelmann disease lead to increased signaling by altering either activation or secretion of the mutant protein. *J Biol Chem* 2003; 278(9):7718-7724.
- (92) Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001; 107(4):513-523.
- (93) Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C *et al.* A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *Am J Hum Genet* 2002; 70(1):11-19.
- (94) Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA *et al.* High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 2002; 346(20):1513-1521.
- (95) Ferrari SL, Deutsch S, Choudhury U, Chevalley T, Bonjour JP, Dermitzakis ET *et al.* Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with variation in vertebral bone mass, vertebral bone size, and stature in whites. *Am J Hum Genet* 2004; 74(5):866-875.
- (96) Koller DL, Ichikawa S, Johnson ML, Lai D, Xuei X, Edenberg HJ *et al.* Contribution of the LRP5 gene to normal variation in peak BMD in women. *J Bone Miner Res* 2005; 20(1):75-80.
- (97) Ferrari SL, Deutsch S, Antonarakis SE. Pathogenic mutations and polymorphisms in the lipoprotein receptor-related protein 5 reveal a new biological pathway for the control of bone mass. *Curr Opin Lipidol* 2005; 16(2):207-214.
- (98) Holmen SL, Giambernardi TA, Zylstra CR, Buckner-Berghuis BD, Resau JH, Hess JF *et al.* Decreased BMD and limb deformities in mice carrying mutations in both Lrp5 and Lrp6. *J Bone Miner Res* 2004; 19(12):2033-2040.
- (99) Akhter MP, Wells DJ, Short SJ, Cullen DM, Johnson ML, Haynatzki GR *et al.* Bone biomechanical properties in LRP5 mutant mice. *Bone* 2004; 35(1):162-169.
- (100) Ai M, Holmen SL, Van Hul W, Williams BO, Warman ML. Reduced affinity to and inhibition by DKK1 form a common mechanism by which high bone mass-associated missense mutations in LRP5 affect canonical Wnt signaling. *Mol Cell Biol* 2005; 25(12):4946-4955.

- (101) Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K *et al.* Targeted disruption of *Cbfa1* results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 1997; 89:755-764.
- (102) Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR *et al.* *Cbfa1*, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell* 1997; 89(5):765-771.
- (103) Quack I, Vonderstrass B, Stock M, Aylsworth AS, Becker A, Brueton L *et al.* Mutation analysis of core binding factor A1 in patients with cleidocranial dysplasia. *Am J Hum Genet* 1999; 65(5):1268-1278.
- (104) Doecke JD, Day CJ, Stephens AS, Carter SL, van Daal A, Kotowicz MA *et al.* Association of functionally different *RUNX2* P2 promoter alleles with BMD. *J Bone Miner Res* 2006; 21(2):265-273.
- (105) Vaughan T, Reid DM, Morrison NA, Ralston SH. *RUNX2* alleles associated with BMD in Scottish women; interaction of *RUNX2* alleles with menopausal status and body mass index. *Bone* 2004; 34(6):1029-1036.
- (106) Vaughan T, Pasco JA, Kotowicz MA, Nicholson GC, Morrison NA. Alleles of *RUNX2/CBFA1* gene are associated with differences in bone mineral density and risk of fracture. *J Bone Miner Res* 2002; 17(8):1527-1534.
- (107) Abu-Amer Y, Erdmann J, Alexopoulou L, Kollias G, Ross FP, Teitelbaum SL. Tumor necrosis factor receptors types 1 and 2 differentially regulate osteoclastogenesis. *J Biol Chem* 2000; 275(35):27307-27310.
- (108) Devoto M, Specchia C, Li HH, Caminis J, Tenenhouse A, Rodriguez H *et al.* Variance component linkage analysis indicates a QTL for femoral neck bone mineral density on chromosome 1p36. *Hum Mol Genet* 2001; 10(21):2447-2452.
- (109) Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M *et al.* Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. *Am J Hum Genet* 2003; 72(1):144-155.
- (110) Spotila LD, Rodriguez H, Koch M, Adams K, Caminis J, Tenenhouse HS *et al.* Association of a polymorphism in the *TNFR2* gene with low bone mineral density. *J Bone Miner Res* 2000; 15(7):1376-1383.
- (111) Albagha OM, Tasker PN, McGuigan FE, Reid DM, Ralston SH. Linkage disequilibrium between polymorphisms in the human *TNFRSF1B* gene and their association with bone mass in perimenopausal women. *Hum Mol Genet* 2002; 11(19):2289-2295.
- (112) Balemans W, Van Wesenbeeck L, Van Hul W. A clinical and molecular overview of the human osteopetroses. *Calcif Tissue Int* 2005; 77(5):263-274.
- (113) Sobacchi C, Vezzoni P, Reid DM, McGuigan FE, Frattini A, Mirolo M *et al.* Association between a polymorphism affecting an AP1 binding site in the promoter of the *TCIRG1* gene and bone mass in women. *Calcif Tissue Int* 2004; 74(1):35-41.
- (114) Pettersson U, Albagha OM, Mirolo M, Taranta A, Frattini A, McGuigan FE *et al.* Polymorphisms of the *CLCN7* gene are associated with BMD in women. *J Bone Miner Res* 2005; 20(11):1960-1967.
- (115) Liu YZ, Liu YJ, Recker RR, Deng HW. Molecular studies of identification of genes for osteoporosis: the 2002 update. *J Endocrinol* 2003; 177(2):147-196.
- (116) Niu T, Chen C, Cordell H, Yang J, Wang B, Wang Z *et al.* A genome-wide scan for loci linked to forearm bone mineral density. *Hum Genet* 1999; 104(3):226-233.
- (117) Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P *et al.* Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. *J Clin Endocrinol Metab* 2000; 85(9):3116-3120.

- (118) Karasik D, Myers RH, Cupples LA, Hannan MT, Gagnon DR, Herbert A *et al.* Genome screen for quantitative trait loci contributing to normal variation in bone mineral density: the Framingham Study. *J Bone Miner Res* 2002; 17(9):1718-1727.
- (119) Deng HW, Xu FH, Huang QY, Shen H, Deng H, Conway T *et al.* A whole-genome linkage scan suggests several genomic regions potentially containing quantitative trait Loci for osteoporosis. *J Clin Endocrinol Metab* 2002; 87(11):5151-5159.
- (120) Karasik D, Cupples LA, Hannan MT, Kiel DP. Age, gender, and body mass effects on quantitative trait loci for bone mineral density: the Framingham Study. *Bone* 2003; 33(3):308-316.
- (121) Kammerer CM, Schneider JL, Cole SA, Hixson JE, Samollow PB, O'Connell JR *et al.* Quantitative trait loci on chromosomes 2p, 4p, and 13q influence bone mineral density of the forearm and hip in Mexican Americans. *J Bone Miner Res* 2003; 18(12):2245-2252.
- (122) Shen H, Zhang YY, Long JR, Xu FH, Liu YZ, Xiao P *et al.* A genome-wide linkage scan for bone mineral density in an extended sample: evidence for linkage on 11q23 and Xq27. *J Med Genet* 2004; 41(10):743-751.
- (123) Peacock M, Koller DL, Fishburn T, Krishnan S, Lai D, Hui S *et al.* Sex-specific and non-sex-specific quantitative trait loci contribute to normal variation in bone mineral density in men. *J Clin Endocrinol Metab* 2005; 90(5):3060-3066.